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Biological Evaluation of Mace for Drug Metabolism Modifying Activity

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Abstract—The single acute treatment of mice with the steam distillate, non-volatile ether extract and methanol extract from mace, arils of *Myristica fragrans*(Myristicaceae) caused a significant prolongation of hexobarbital-induced narcosis, an increase in strychnine toxicity as well as a significant decrease in hepatic microsomal drug metabolizing enzyme activities. On seven daily consecutive administrations, however, the duration of narcosis was markedly shortened and significant increases in the hepatic enzyme activities were shown. From the non-volatile ether fraction, macelignan, a new lignan, mp 70~72° was isolated as an active principle.

Keywords—*Myristica fragrans* • Myristicaceae • macelignan • drug metabolizing enzyme modifier

Mace is dried arillodes of ripe seeds of *Myristica fragrans*(Myristicaceae), an evergreen tree indigenous to the Molucca Island, which is now widely cultivated in the South-East Asian regions.

Mace has been used not only for aromatic stomachics in traditional Chinese medicine but for flavor, spice and condiment. In a course of series of biological screening of medicinal plants and spices, it was found that the methanol extract of nutmeg and the ether extract of mace caused a marked alteration in hexobarbital(HB)-induced narcosis suggesting the presence of hepatic drug metabolizing enzyme(DME) modifiers in this plant.[2,3] In this paper, we describe the biological evaluation of various fractions of mace for alteration in hepatic DME activities as well as isolation of a new lignan as one of active principles in this plant.

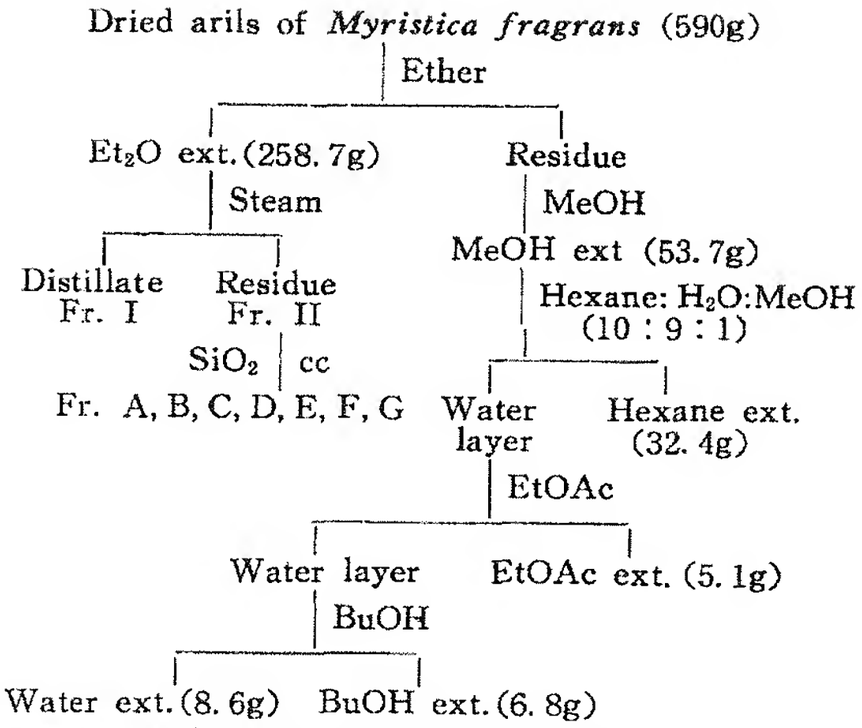
* Part 8 in the series: Studies on crude drugs acting on drug-metabolizing enzymes. For part 7 see ref. (1).

Experimental methods

Materials—The dried mace was supplied from a market in Bangkok, Thailand. SKF-525 A was a gift from Smith, Kline and French, Philadelphia, P.A. Phenobarbital was USP grade and NADP, glucose-6-phosphate were purchased from Sigma Chemical Co. All other chemicals were of first grade commercially available.

Physico-chemical methods—Melting point was taken on Mitamura Riken(MRK 4204) melting point apparatus and is uncorrected. IR was taken in KBr pellet with Perkin-Elmer (281B) IR spectrometer, NMR with Varian FT-80A NMR spectrometer, MS with Hewlett Packard (5985B) GC/MS spectrometer, and optical rotation with Ameresco Autopolarimeter.

Extraction and fractionation—The dried mace were coarsely powdered and extracted with



Scheme 1. Extraction and fractionation of mace.

ether and the marc after ether extraction was extracted with 95% methanol.

The two extracts were further fractionated into steam distillate (Fraction I), non-volatile ether extract, hexane, ethylacetate and butanol fractions as illustrated in Scheme 1.

The non-volatile ether extract (Fraction II) was subjected to column chromatography on silica gel (Kiesel gel 60G, E. Merck) using benzene and ether (gradient) as an eluent to give 7 fractions (Fr. A–G).

Isolation of macelignan—From fraction A a lignan was obtained as a pale yellow oil, which on recrystalization from n-hexane: ether gave colorless prisms, mp. 70~72°, $[\alpha]_D^{20}=+5.28°$ (c, 1.8 in $CHCl_3$). $FeCl_3$ in ethanol, green color; Gibbs and Emerson test, negative.

$UV_{\lambda max}^{MeOH}$ nm (logε): 213(3.97), 230(3.88), 285(3.77); $IR_{\nu max}^{KBr}$ cm^{-1}: 3480(OH), 1852, 1740, 1610, 1514, 1500, 1486, 1440 (aromatic), 1376 1250, 1028, 926(O-CH_2-O); PMR(80 MHz, δ in $CDCl_3$): 0.84(6H, d, J=6.6 Hz, 2×CH_3-), 1.5~1.85(2H, m, 2×CH-), 5.42(H, s, exchangable with D_2O, OH), 5.91(2H, s, -O-CH_2-O-), 6.59~6.81(6H, m, 2×aromatic H); MS (m/z, relative intensity): 328(M^+, 11), 137 ($C_8H_9O_2^+$, 100), 135($C_8H_7O_2^+$, 68).

Measurement of Hexobarbital-induced sleeping time—The experiment was carried out in two phases. In the first phase experiment, male dd mice weighing 17~25g were pretreated with a single ip administration of the test materials 30min prior to the measurement of HB-induced sleeping time. In the second phase experiment, mice were pretreated with 7 consecutive daily po administrations of the materials forty eight hr prior to the measurement of the sleeping time.

Mice were observed for sleep as evidenced by loss of the righting reflex. The duration of sleeping time was evaluated from the time of loss to the time the animals regained the righting reflex.

Measurement of strychnine mortality—The materials were given 30min prior to the administration of strychnine nitrate.

The dose of ST (1.1mg/kg, ip) caused 30% mortality within 30min in untreated mice. The mice were observed for 30min and the mortality was recorded.

Enzyme assays—The oxidative metabolism of hexobarbital, aminopyrine, and aniline and cytochrome p-450 concentration in microsomal fractions were determined as described previously.[1]

Result and Discussion

Table I shows the results of the effect of the ether extract and methanol extract obtained from the marc after ether extraction on hepatic DME function as measured by the duration of HB-induced sleeping time and ST-mortality in mice.

It was shown that in the first phase experiment, both extracts caused a significant prolongation of HB-induced sleeping time at a dose of 200mg/kg intraperitoneally, although the potencies were weaker than that of SKF-525A, a well known typical enzyme inhibitor.[4] This prolongation of sleeping time was not considered

Table I. Effects of the ether extract and methanol extract of mace on hexobarbital-induced hypnosis and strychnine mortality in mice

Treatment	Dose (mg/kg)	Hexobarbital hypnosis						Strychnine mortality[c] (No. died/ No. used)
		Phase I[a]			Phase II[b]			
		Number of mice	Sleeping time (min.±S.E.)	Percent of control	Number of mice	Sleeping time (min.±S.E.)	Percent of control	
Control	0.5% CMC	6	23.5±1.6	—	6	65.2±5.1	—	3/10
SKF-525A	30	6	178.4±14.3**	759.3	6	—	—	10/10
Phenobarbital-Na	50	6	—	—	6	22.3±2.2**	34.4	—
Et_2O ext.	200	6	50.6±5.4**	215.5	6	37.1±2.1**	57.1	9/10
MeOH ext.	200	6	42.9±3.2**	182.6	6	41.5±3.0*	63.7	7/10

a) A single i.p. treatment 30min before injection of HB (50mg/kg, i.p.).
b) Seven consecutive daily oral treatments 48hr before injection of HB (100mg/kg, i.p.).
c) A single i.p. treatment 30min before injection of strychnine nitrate (1.10mg/kg, i.p.).
Significantly different from the control; *$p<0.01$ **$p<0.001$

Table II. Effects of a single treatment with the ether extract and methanol extract on DME activities in mice

DME	Treatment			
	Control (0.5% CMC)	SKF-525A (30mg/kg, i.p.).	Et_2O ext. (200mg/kg, i.p.)	MeOH ext. (200mg/kg, i.p.)
Aminopyrine N-demethylase (μ moles/g prot./min)	0.520±0.023	0.213±0.020** (59.0)	0.256±0.050** (50.7)	0.253±0.043** (51.3)
Hexobarbital hydroxylase (μ moles/g prot./min)	0.440±0.025	0.215±0.025** (51.1)	0.276±0.028* (37.3)	0.295±0.076* (33.0)
Aniline hydroxylase (μ moles/g prot./min)	0.177±0.006	—	0.066±0.006* (62.7)	0.090±0.006* (49.2)

Animals were killed 30min after a single treatment.
Each data represents mean±S.E. of 3 separate determinations.
Figures in parentheses indicate % inhibition.
Significatly different from control; *$p<0.05$, **$p<0.01$.

to be due to a CNS-depressant action but due to inhibition of hepatic drug metabolism because the single treatment of the extracts also increased toxicity of ST which is metabolized enzymatically in hepatic DME system.[5]

On the other hand, these extracts significantly shortened the duration of sleeping time in the second phase experiment which was carried out 48hr after the last of 7 daily consecutive treatments. From above results, it was presumed that the mace extracts had a biphasic response on hepatic DME system, both inhibitory and inducing effects.

In order to confirm this assumption, direct effect of the mace extracts on the metabolism of some classical substrates such as aminopyrine, hexobarbital, and aniline in both phases was evaluated. In accord with the result on the barbiturate-induced sleeping time, the enzyme activities of livers 30min after a single treatment of both extracts were significantly reduced; 50.7 and 51.3% in aminopyrine N-demethylase;37.3 and 33% in hexobarbital hydroxylase; 62.7 and 49.2% in aniline hydroxylase respectively compared to those of the corresponding controls (Table II).

As shown in Table III, on repeated treatments with the extracts, however, aminopyrine N-demethylase and hexobarbital hydroxylase activities were significantly elevated by 67.9% and

Table III. Effects of repeated treatments with mace ether extract and methanol extract on DME activities and cytochrome p-450 content in mice

DME	Treatment[a]			
	Control (0.5% CMC)	Phenobarbital (50mg/kg/day, p.o.)	Et_2O ext. (200mg/kg/day, p.o.)	MeOH ext. (200mg/kg/day, p.o.)
Aminopyrine N-demethylase (μmoles/g prot./min)	0.290±0.023	0.557±0.030*** (192.1)	0.487±0.066** (167.9)	0.440±0.036** (151.7)
Hexobarbital hydroxylase (μmoles/g prot./min)	0.365±0.011	0.856±0.035 (234.5)	0.597±0.056** (163.6)	0.575±0.093* (157.5)
Aniline hydroxylase (μmoles/g prot./min)	0.136±0.006	0.206±0.010** (151.5)	0.133±0.013 (97.8)	0.140±0.003 (102.9)
Cyt. p-450 (μmoles/mg prot.)	0.76±0.06	1.69±0.03*** (222.4)	0.95±0.02* (125.0)	0.92±0.05 (121.0)

a) Seven consecutive daily treatments 48 hr before test.
Data represent mean±S.E. of 3 separate determinations.
Figures in parentheses indicate % of control.
Significantly different from control: *$p<0.05$, **$p<0.02$, ***$p<0.01$.

Table IV. Effects of the various fractions of mace on hexobarbital induced hypnosis and strychnine mortality

Treatment	Hexobarbital Hypnosis						Strychnine mortality[c] (No. died/ No. used)
	Phase I[a]			Phase II[b]			
	Number of mice	Sleeping time (min.±S.E.)	Percent of control	Number of mice	Sleeping time (min.±S.E.)	Percent of control	
Control	5	25.0±3.7	—	5	72.2±4.5	—	3/10
Fr. I	5	69.7±2.4***	278.7	5	30.8±2.5***	42.7	8/10
Fr. II	5	51.4±3.2***	205.6	5	52.0±6.1**	72.0	9/10
Hexane ext	5	61.8±2.5***	247.3	5	47.0±4.1**	65.1	6/10
EtOAc ext	5	47.4±8.6*	189.6	5	47.8±1.3**	66.2	—
BuOH ext	5	24.1±5.3	96.5	5	67.4±3.7	93.4	—
H_2O ext	5	40.0±3.0*	160.1	5	57.3±2.7*	79.4	—

a) A single treatment (200mg/kg, i.p.) 30 min before injection of HB (50mg/kg, i.p.).
b) Seven consecutive daily treatments (200mg/kg, p.o.) 48 hr before injection of HB (100mg/kg, i.p.).
c) A single treatment (200mg/kg, i.p.) 30min before injection of strychnine nitrate(1.10mg/kg, i.p.).
Significantly different from the control; *$p<0.05$, **$p<0.01$, ***$p<0.001$

63.6% in the case of the ether extract and 51.7 % and 57.5% in the case of the methanol extract, respectively compared to those of the corresponding control, although aniline hydroxylase activity was unaffected, which was somewhat different from phenobarbital treatment which caused a significant elevation of the activity.

The level of cytochrome p-450 which is a terminal enzyme in DME system was also significantly increased.

Based on the above results, we attempted a further systematic fractionation of the extracts (Scheme 1) monitoring by HB-induced sleeping time and ST-mortality tests, in order to isolate active principle(s).

As shown in Table IV, the steam distillate (Fr. I), non-volatile ether extract (Fr. II) and hexane fraction were shown to cause a distinct prolongation of HB-induced hypnosis and potentiation of ST-mortality in the first phase experiment and a significant shortening of the barbiturate action in the second phase experiment

Table V. Effects of column chromatographic subfractions from the non-volatile ether extract on hexobarbital-induced hypnosis and strychnine mortality

Treatment	Dose (mg/kg, i.p.)	Hexobarbital hypnosis			Strychnine mortality[b] (No. died/ No. used)
		Number of mice	Sleeping time[a] (min±S.E.)	Percent of control	
Control	—	5	27.5±2.5	—	3/10
Fr. A	100	5	86.9±4.8***	316.0	7/10
Fr. B	100	5	38.4±3.7*	139.6	—
Fr. C	100	5	32.8±3.9	119.3	—
Fr. D	100	5	61.0±8.6**	221.8	6/10
Fr. E	100	5	51.3±3.0***	186.5	7/10
Fr. F	200	5	31.4±7.6	114.2	—
Fr. G	100	5	24.5±2.3	90.0	—

Mice were treated with each fraction 30 min prior to the injection of a) HB (50mg/kg, i.p.) and b) strychnine nitrate (1.10mg/kg, i.p.).
Significantly different from the control; *$p<0.05$, **$p<0.01$, ***$p<0.001$

(Table IV).

Among them, the non-volatile ether extract (Fr. II) was subjected to SiO_2 column chromatography to give seven fractions (Fr. A-G) which were bioassayed for potentiating effect on HB-induced narcosis and ST-mortality in mice.

As shown in Table V, the fraction A was found to be the most active, from which a new lignan was isolated.

This lignan was named macelignan and the structure elucidation will be described elsewhere.[6]

Macelignan with a single treatment at a dose

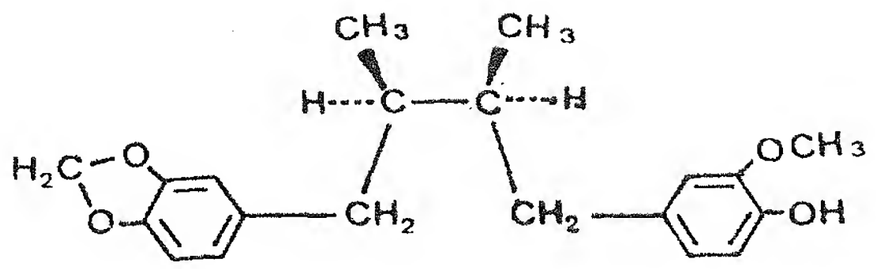


Fig. 1. Structure of macelignan.

of 100mg/kg ip demonstrated to cause a significant prolongation of HB-induced sleeping time by 222.9% compared to that of the control, increase in ST-mortality as well as exhibited a significant inhibitory activity on aminopyrine

Table VI. Effects of macelignan on hexobarbital-induced hypnosis, strychnine mortality and aminopyrine metabolism *in vitro*

Treatment	Hexobarbital hypnosis[a] (min±S.E.)	Strychnine mortality[b] (No. died/No. used)	Aminopyrine N-demethylase[e] (μmoles/min/g prot.)
Control	27.5±2.5(5)[c]	3/10	0.513±0.030
Macelignan	88.8±5.7(5)*	7/10	0.347±0.010*
(100mg/kg, i.p.)	(322.9)[d]	—	(67.5)[d]

Mice were treated 30min before injection of a) HB(50mg/kg, i.p.) and b) Strychnine nitrate (1.10mg/kg, i.p.).
c) Number of mice.
d) Percent of the control.
e) Enzyme activity was measured *in vitro* on 10,000g supernatant fraction of male Sprague Dawley rat liver. Substrate concentration, 0.82 mM; inhibitor concentration, 0.25mM. Data are mean±S.E. of triplicate determinations.
Significantly different from the control: *$p<0.001$.

metabolism *in vitro* (Table VI).

It has been previously demonstrated that a number of alkylmethoxy benzene and alkylmethylenedioxy benzene essential oils including myristicin, one of main volatile essential oil components not only of nutmeg but mace caused a significant potentiation of sodium pentobarbital-induced sleeping time in mice suggesting their inhibitory activity of microsomal drug metabolizing enzymes.[7] It could therefore be attributable that a significant alteration caused by the steam-distillate rich in volatile essential oils are partly due to a key component, myristicin.

Although the precise mode of the activity of the macelignan is not yet known at present, the methylenedioxy group in the lignan could be suggested to play an important role in excerting the inhibitory activity, based on the facts that some methylenedioxy benzene compounds such as piperine, piperonylbutoxide, sesamex, and tropital significantly inhibited hepatic oxidative xenobiotic metabolism.[8~10]

The assumption is further supported by the finding that dihydroguaiaretic acid which is devoid of the methylenedioxy group failed to produce any significant effect on hepatic DME activity (Data not shown).

Further studies on isolation of other active components and on the detailed mode of the activity are in progress.

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